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# Fluid and Protein Secretion by the Submandibular Glands of Weanling Rats in Response to Cholinergic and Peptidergic Agonists at Various Doses

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## Abstract

Fluid and protein secretion by the submandibular glands of 25-day-old rats were examined and compared in response to three cholinergic and four peptidergic sialogogues at various doses.

All cholinergic and peptidergic agonists used were potent sialogogues for the submandibular glands of the weanling rats over the wide range of doses used. The cholinergic agonists, bethanechol and methacholine and the peptidergic agonists, substance P, substance $P^{Tyr8}$ and eledoisin-related peptide used intravenously, acted similarly to each other on the submandibular glands of the rats, late in the natural weaning period, but carbachol and physalaemin had slightly different effects. Of the peptidergic agonists, physalaemin was the most potent sialogogue among four tachykinins tested at the low dose. The types of protein secreted by the submandibular glands of the weanling rats in response to all sialogogues used here were typical of the $\beta$-type.

These results indicate that all agonists used could mainly stimulate the acinar cells of the submandibular glands of the weanling rats which have already fully developed functionally at this time.

Biochemical and morphological changes in rat submandibular glands during neo- and post-natal development have been extensively elucidated by many investigators (Sreebny et al 1956; Bylund et al 1982; Abe et al 1987; Ball et al 1991; Moreira et al 1991). Several types of cells with distinctive secretory granules are transiently present in the secretory end pieces of these glands during the perinatal period (Chang 1974; Cutler & Chaudhry 1975; Zajicek et al 1985). These cells are gradually replaced by mature acinar cells during the first five weeks of post-natal development.

Rats deprived of submandibular–sublingual saliva before the 10th day of age die of starvation, but they are able to suckle effectively if the submandibular–sublingual ablation is not done until the 13th day or later, when the development of the parotid glands has begun (Plagge 1938). However, effective suckling can be restored to the desalivated pups by periodic treatment of their lips with vaseline (Epstein et al 1970). Therefore, saliva is important as a sealant of the pup's lips and mouth to the maternal nipple and is thus essential for feeding throughout the suckling period (Epstein et al 1970).

Little information is, however, available on fluid or salivary protein secretion by the submandibular glands during this important weaning period. Some information is available on fluid or protein secretion by the submandibular glands of older rats, in response to cholinergic (Abe & Dawes 1978; Oikawa 1983) and peptidergic agonists (Emmelin & Lenninger 1967; Schneyer & Hall 1968; Martinez & Martinez 1981; Yu et al 1983; Fleming et al 1984).

The present study was designed to determine whether the submandibular glands of weanling rats responded to three cholinergic and four peptidergic agonists at different doses in the same manner as do glands from adult animals.

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## Materials and Methods

*Saliva collection*

Male Sprague-Dawley rats at 3·5 weeks of age, during the weaning period, were distributed among experimental groups consisting of from 5 to 7 animals each. A 12-h light/dark cycle was maintained by an automatic timing device, with lights turned off at 1800 h and the animals had free access to rat chow (MF solid rat chow, Oriental Kobokogyo, Tokyo) and water.

The rats were deprived of food from 1700 to 0900 h before the collection of saliva to minimize the circadian rhythms of feeding activity, submandibular secretion and storage of proteins as complicating factors (Dawes 1972), then anaesthetized with sodium pentobarbitone (Nembutal, 50 mg $kg^{-1}$), secured in a supine position with tape, and tracheotomized to facilitate respiration during the saliva collection. Both submandibular ducts were cannulated intra-orally by the method of Yoshida et al (1967). With the exception of an initial drop, which was discarded, submandibular saliva was collected for 1 h for intraperitoneal or 15 min for intravenous administration into Pyrex glass tubes kept in ice and volumes were estimated by weight, assuming the specific gravity of saliva to be 1·0. As secretory stimuli, the cholinergic agonists were methacholine (1, 1·5, 2·5, 3·5 and 5 mg

$kg^{-1}$ injected intraperitoneally at 30-min intervals to give a final dose of 2, 3, 5, 7 and 10 mg $kg^{-1}$, Sigma, St Louis, USA), carbachol (10, 20, 25, 50, 75 and 100 $\mu g\ kg^{-1}$, Sigma) and bethanechol (0·5, 0·75, 1, 2 and 5 mg $kg^{-1}$, Sigma). To compare with the types of protein secreted by the submandibular glands during the weaning period, pilocarpine (Nacalai Tesque, Kyoto), a mixed cholinergic and adrenergic agonist (Schneyer & Hall 1966), was used at a dose of 8 mg $kg^{-1}$ as a control. The peptidergic agonists (Peptide Institute, Osaka) used were substance P (0·5, 1, 2, 3 and 5 mg $kg^{-1}$), substance $P^{Tyr8}$ (0·5, 1, 2, 3 and 5 mg $kg^{-1}$), physalaemin (0·005, 0·01, 0·015, 0·02, 0·025, 0·05, 0·1, 0·25, 0·5, 1, 2 and 3 mg $kg^{-1}$) and eledoisin-related peptide (0·5, 1, 3 and 5 mg $kg^{-1}$, i.v.). All drugs were given by intraperitoneal injection except for eledoisin-related peptide. After saliva collection, the sublingual glands were carefully dissected away from the submandibular glands and the latter were removed and weighed. The gland weight was used in the calculation of the rate of salivary flow.

*Saliva analyses*

Saliva samples were analysed for total protein by the method of Lowry et al (1951), with casein as a standard, and for esteroprotease activities by the method of Walsh (1970). *p*-Tosyl L-arginine methylester (Sigma) was used as a substrate, assayed for 5 min at 25°C and measured at 247 nm. The types of proteins present in submandibular saliva were analysed by iso-electric focusing (IEF) by the PhastSystem (Pharmacia, Sweden) on both pH 3·5–5 and pH 3·5–9 gels as described previously (Tanaka et al 1990; Okina et al 1993) and these were subsequently stained with silver (Heukeshoven & Dernick 1985). The same amounts (2 $\mu g$) of protein were added to each lane in a given gel.

*Statistical test*

Statistical analysis was by factorial analysis of variance. When this gave a significant F value, Duncan's new multiple range test was carried out on the adjusted means.

## Results

For submandibular saliva elicited by intraperitoneal injection of bethanechol, the volumes of saliva secreted progressively increased with increasing doses up to 2 mg $kg^{-1}$, but were not significantly higher with a higher dose (Table 1). Doses at 2 and 5 mg $kg^{-1}$ of bethanechol were nearly lethal. With methacholine, however, the volumes secreted peaked with a dose of 5 mg $kg^{-1}$ but fell when higher doses were used (Table 2). With carbachol, the saliva volumes progressively increased with increasing doses up to 100 $\mu g\ kg^{-1}$ (Table 3). A dose of 100 $\mu g\ kg^{-1}$, however, was almost lethal. At a dose of 10 $\mu g\ kg^{-1}$, no salivation occurred in response to carbachol.

The concentrations of protein in saliva elicited by the three cholinergic agonists were very low and were significantly different at only a few of the doses used (bethanechol, 1 mg $kg^{-1}$; methacholine, 5 mg $kg^{-1}$; and carbachol, 20 and 25 $\mu g\ kg^{-1}$; Tables 1–3). The total output of protein secreted in response to methacholine was very low and was significantly different only at a few of the doses used (5 and 7 mg $kg^{-1}$). With bethanechol and carbachol it increased at the higher doses, as shown in Tables 1 and 3, respectively.

Table 1. The effects of bethanechol at different intraperitoneal doses on submandibular salivary flow rates and protein secretion (means ± s.e.).

| | Dose (mg $kg^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 0·5 | 0·75 | 1 | 2 | 5 |
| Volume of saliva ($\mu$L in 1 h) | $38·2^{a}$ ± 3·4 | $72·7^{a}$ ± 6·7 | $122·0^{a,b}$ ± 3·1 | $199·4^{b}$ ± 22·2 | $186·4^{b}$ ± 11·9 |
| Flow rate (nL $mg^{-1}$ $min^{-1}$) | $9·4^{a}$ ± 0·7 | $16·4^{a}$ ± 1·5 | $30·1^{a,b}$ ± 1·2 | $46·1^{b}$ ± 3·0 | $62·1^{b}$ ± 2·8 |
| Protein concn (mg $dL^{-1}$) | $89·3^{a}$ ± 10·4 | $87·7^{a}$ ± 16·1 | $43·7^{b}$ ± 6·0 | $62·1^{b,c}$ ± 8·5 | $83·3^{a,c}$ ± 5·3 |
| Protein secreted (mg $h^{-1}$) | $0·03^{a}$ ± 0·003 | $0·06^{a,b}$ ± 0·01 | $0·05^{a}$ ± 0·01 | $0·12^{b,c}$ ± 0·02 | $0·15^{c}$ ± 0·01 |
| Protease activity (units $h^{-1}$) | $0·05^{a}$ ± 0·01 | $0·08^{a}$ ± 0·01 | $0·16^{b}$ ± 0·02 | $0·16^{b}$ ± 0·04 | $0·15^{b}$ ± 0·01 |
| (units (mg protein)$^{-1}$) | $1·39^{a}$ ± 0·10 | $1·58^{a,b}$ ± 0·19 | $1·82^{b}$ ± 0·19 | $1·40^{a}$ ± 0·22 | $0·98^{c}$ ± 0·07 |
| No. of glands | 6 | 9 | 7 | 7 | 4 |

Means with different superscripts (a–c) are significantly different by Duncan's new multiple range test ($P < 0·05$).

The relative (units $h^{-1}$) and specific (units (mg protein)$^{-1}$) activities of esteroprotease in salivas elicited by the three cholinergic agonists were significantly higher at the higher doses or at the doses which elicited the maximum secretory responses (Tables 1–3). However, the specific activities of esteroprotease in saliva elicited with methacholine were significantly higher at the doses which elicited the maximum secretory responses (Table 2).

Table 2. The effects of methacholine at different intraperitoneal doses on submandibular salivary flow rates and protein secretion (means ± s.e.).

| | Dose (mg $kg^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 5 | 7 | 10 |
| Volume of saliva ($\mu$L in 1 h) | $18·7^{a}$ ± 4·4 | $73·0^{a}$ ± 8·0 | $191·6^{b}$ ± 8·3 | $190·2^{b}$ ± 6·2 | $54·1^{a}$ ± 2·2 |
| Flow rate (nL $mg^{-1}$ $min^{-1}$) | $4·9^{a}$ ± 1·1 | $19·5^{a}$ ± 2·1 | $49·8^{b}$ ± 2·7 | $43·3^{b}$ ± 2·5 | $15·2^{a}$ ± 0·5 |
| Protein concn (mg $dL^{-1}$) | $98·8^{a,b}$ ± 14·4 | $114·0^{b,c}$ ± 18·3 | $129·3^{c}$ ± 23·0 | $74·1^{a}$ ± 15·5 | $87·7^{a,b}$ ± 9·5 |
| Protein secreted (mg $h^{-1}$) | $0·03^{a}$ ± 0·01 | $0·08^{a}$ ± 0·01 | $0·23^{b}$ ± 0·04 | $0·25^{b}$ ± 0·09 | $0·10^{a}$ ± 0·03 |
| Protease activity (units $h^{-1}$) | $0·07^{a}$ ± 0·003 | $0·10^{a,b}$ ± 0·03 | $0·25^{b,c}$ ± 0·03 | $0·39^{c}$ ± 0·23 | $0·21^{a,b}$ ± 0·07 |
| (units (mg protein)$^{-1}$) | $1·88^{a}$ ± 0·08 | $1·27^{b}$ ± 0·35 | $1·29^{b}$ ± 0·18 | $1·40^{b}$ ± 0·32 | $1·99^{a}$ ± 0·33 |
| No. of glands | 5 | 5 | 8 | 6 | 4 |

Means with different superscripts (a–c) are significantly different by Duncan's new multiple range test ($P < 0·05$).

Table 3. The effects of carbachol at different intraperitoneal doses on submandibular salivary flow rates and protein secretion (means ± s.e.).

| | Dose ($\mu$g kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 20 | 25 | 50 | 75 | 100 |
| Volume of saliva ($\mu$L in 1 h) | 34·9$^{a}$ ± 2·5 | 35·0$^{a}$ ± 3·6 | 98·4$^{a,b}$ ± 5·6 | 210·1$^{b,c}$ ± 21·7 | 259·6$^{c}$ ± 9·8 |
| Flow rate (nL mg$^{-1}$ min$^{-1}$) | 9·1$^{a}$ ± 0·6 | 9·4$^{a}$ ± 0·8 | 26·8$^{a,b}$ ± 1·5 | 56·1$^{b,c}$ ± 6·5 | 67·7$^{c}$ ± 3.5 |
| Protein concn (mg dL$^{-1}$) | 191·2$^{a}$ ± 21·1 | 159·8$^{a}$ ± 18·9 | 58·8$^{b}$ ± 5·5 | 44·9$^{b}$ ± 5·5 | 57·3$^{b}$ ± 5·8 |
| Protein secreted (mg h$^{-1}$) | 0·06$^{a}$ ± 0·01 | 0·06$^{a}$ ± 0·01 | 0·06$^{a}$ ± 0·001 | 0·09$^{a}$ ± 0·02 | 0·15$^{b}$ ± 0·01 |
| Protease activity (units h$^{-1}$) | 0·08$^{a,c}$ ± 0·004 | 0·06$^{a}$ ± 0·001 | 0·15$^{b,c}$ ± 0·001 | 0·20$^{b}$ ± 0·03 | 0·19$^{b}$ ± 0·002 |
| (units (mg protein)$^{-1}$) | 1·43$^{a,c}$ ± 0·19 | 1·13$^{a}$ ± 0·26 | 3·12$^{b}$ ± 0·39 | 2·46$^{b,c}$ ± 0·31 | 3·10$^{b}$ ± 0·10 |
| No. of glands | 5 | 5 | 14 | 5 | 3 |

Means with different superscripts (a–c) are significantly different by Duncan's new multiple range test ($P < 0·05$).

The types of protein in saliva secreted in response to the three cholinergic agonists and to pilocarpine at all doses tested were typical of the $\beta$-type on both pH 3·5–5 and 3·5–9 gels stained with silver (data not shown).

For submandibular saliva elicited by intraperitoneal injection of substance P, substance P$^{Tyr8}$ and eledoisin-related peptide, the volumes of saliva secreted and flow rates progressively increased with increasing doses up to 3 mg kg$^{-1}$ but fell when a higher dose was used (Tables 4, 5, 7). However, the volumes of saliva secreted and flow rates in response to physalaemin peaked with a dose of 0·02 mg kg$^{-1}$ but were progressively reduced with higher doses (Table 6).

Table 4. The effects of substance P at different intraperitoneal doses on submandibular salivary flow rates and protein secretion (means ± s.e.).

| | Dose (mg kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 0·5 | 1 | 2 | 3 | 5 |
| Volume of saliva ($\mu$L in 1 h) | 29·7$^{a}$ ± 2·0 | 160·2$^{b}$ ± 12·1 | 174·8$^{b}$ ± 9·5 | 213·9$^{b}$ ± 23·3 | 179·6$^{b}$ ± 8·8 |
| Flow rate (nL mg$^{-1}$ min$^{-1}$) | 7·5$^{a}$ ± 0·6 | 39·4$^{b}$ ± 3·4 | 44·8$^{b}$ ± 2·1 | 52·9$^{b}$ ± 6·9 | 52·6$^{b}$ ± 2·6 |
| Protein concn (mg dL$^{-1}$) | 136·8$^{a}$ ± 11·6 | 132·3$^{a}$ ± 5·5 | 82·7$^{b}$ ± 7·4 | 122·9$^{a}$ ± 7·2 | 154·0$^{a}$ ± 5·0 |
| Protein secreted (mg h$^{-1}$) | 0·04$^{c}$ ± 0·003 | 0·21$^{a,b}$ ± 0·01 | 0·14$^{b,c}$ ± 0·02 | 0·26$^{a,b}$ ± 0·04 | 0·28$^{a}$ ± 0·02 |
| Protease activity (units h$^{-1}$) | 0·06$^{b}$ ± 0·01 | 0·09$^{b}$ ± 0·01 | 0·10$^{b}$ ± 0·01 | 0·12$^{a,b}$ ± 0·03 | 0·17$^{a}$ ± 0·01 |
| (units (mg protein)$^{-1}$) | 1·50$^{a}$ ± 0·24 | 0·47$^{b}$ ± 0·05 | 0·75$^{b}$ ± 0·12 | 0·41$^{b}$ ± 0·05 | 0·64$^{b}$ ± 0·06 |
| No. of glands | 6 | 8 | 9 | 8 | 6 |

Means with different superscripts (a–c) are significantly different by Duncan's new multiple range test ($P < 0·05$).

Table 5. The effects of substance p$^{Tyr8}$ at different intraperitoneal doses on submandibular salivary flow rates and protein secretion (means ± s.e.).

| | Dose (mg kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 0·5 | 1 | 2 | 3 | 5 |
| Volume of saliva ($\mu$L in 1 h) | 103·3$^{a}$ ± 7·3 | 168·4$^{b}$ ± 29·6 | 175·6$^{b}$ ± 7·66 | 205·2$^{b}$ ± 12·6 | 167·0$^{b}$ ± 6·1 |
| Flow rate (nL mg$^{-1}$ min$^{-1}$) | 25·5$^{a}$ ± 2·2 | 32·6$^{a,b}$ ± 5·5 | 55·2$^{c}$ ± 2·7 | 63·0$^{c}$ ± 4·3 | 49·9$^{b,c}$ ± 2·1 |
| Protein concn (mg dL$^{-1}$) | 69·0$^{a}$ ± 7·8 | 79·4$^{a,b}$ ± 7·3 | 142·9$^{c}$ ± 11·2 | 136·0$^{b,c}$ ± 11·1 | 180·1$^{c}$ ± 12·1 |
| Protein secreted (mg h$^{-1}$) | 0·07$^{a}$ ± 0·01 | 0·13$^{a,b}$ ± 0·03 | 0·25$^{b,c}$ ± 0·02 | 0·27$^{c}$ ± 0·02 | 0·30$^{c}$ ± 0·02 |
| Protease activity (units h$^{-1}$) | 0·10$^{b}$ ± 0·02 | 0·10$^{b}$ ± 0·02 | 0·11$^{a,b}$ ± 0·01 | 0·13$^{a}$ ± 0.01 | 0·10$^{b}$ ± 0·01 |
| (units (mg protein)$^{-1}$) | 1·71$^{a}$ ± 0·24 | 0·77$^{b}$ ± 0·09 | 0·46$^{b}$ ± 0·05 | 0·47$^{b}$ ± 0·03 | 0·34$^{b}$ ± 0·07 |
| No. of glands | 7 | 5 | 10 | 10 | 6 |

Means with different superscripts (a–c) are significantly different by Duncan's new multiple range test ($P < 0·05$).

The concentrations of protein in saliva elicited by substance P$^{Tyr8}$ and physalaemin were significantly higher with high doses (Tables 5, 6), but in saliva elicited by substance P and eledoisin-related peptide there was little effect of dose (Tables 4, 7). The total output of protein progressively increased with increasing dose in saliva secreted in response to substance P$^{Tyr8}$, physalaemin and eledoisin-related peptide (Tables 5, 6, 7), whereas it was not significantly changed in saliva secreted in response to substance P (Table 4).

The relative and specific activities of esteroprotease in salivas elicited by substance P, substance P$^{Tyr8}$, physalaemin and eledoisin were relatively low and were not significantly changed with increasing doses (Tables 4, 5, 6, 7). However, in saliva elicited by the four peptidergic agonists except for eledoisin-related peptide at the lowest doses used, the specific activities of esteroprotease were significantly higher (Tables 4, 5, 6).

The types of protein in salivas secreted in response to the four peptidergic agonists were also typical of the $\beta$-type on both pH 3·5–5 and 3·5–9 gels stained with silver (data not shown).

## Discussion

There have been no reports, so far, on the types of proteins secreted by the submandibular glands of 25-day-old rats in response to various agonists used here. However, there are several reports on fluid, electrolyte and protein secretion by submandibular glands of adult rats in response to substance P, physalaemin, bethanechol, methacholine and carbachol at different doses given intraperitoneally and intravenously (Abe & Dawes 1978, 1982; Martinez & Martinez 1981; Yu et al 1983; Ekström et al 1983; Iwabuchi et al 1986, 1988; Bobyock et al 1986; Tumilasci et al 1986; Ueha et al 1989; Sugisawa & Takai 1991) or in in-vitro experiments (Fleming et al 1984). There have been no reports, to our knowledge,

Table 6. The effects of physalaemin at different intraperitoneal doses on submandibular salivary flow rates and protein secretion (means ± s.e.).

| | Dose (mg kg$^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0·005 | 0·01 | 0·015 | 0·02 | 0·025 | 0·05 | 0·1 | 0.25 | 0·5 | 1·0 | 2·0 | 3·0 |
| Volume of saliva | 25·0$^{a}$ | 60·4$^{a}$ | 191·5$^{b,c}$ | 231·6$^{b}$ | 161·3$^{c,d}$ | 173·4$^{c,d}$ | 164·1$^{c,d}$ | 156·2$^{c,d}$ | 147·3$^{c,d}$ | 154·3$^{c,d}$ | 148·7$^{c,d}$ | 140·9$^{d}$ |
| ($\mu$L in 1 h) | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 3·7 | 4·7 | 11·9 | 14·6 | 4·4 | 15·7 | 13·3 | 7·6 | 14·7 | 6·2 | 12·0 | 19·9 |
| Flow rate | 6·0$^{a}$ | 15·4$^{a}$ | 51·9$^{c,d}$ | 70·7$^{b}$ | 44·9$^{c,d}$ | 58·0$^{b,c}$ | 55·1$^{c,d}$ | 49·4$^{c,d}$ | 41·9$^{d}$ | 44·0$^{c,d}$ | 40·1$^{d}$ | 41·6$^{d}$ |
| (nL mg$^{-1}$ min$^{-1}$) | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 0·9 | 1·2 | 3·8 | 8·0 | 1·3 | 6·0 | 4·1 | 1·3 | 5·4 | 1·2 | 3·4 | 5·4 |
| Protein concn | 167·9$^{a,b}$ | 99·7$^{c,d}$ | 74·1$^{c,d}$ | 46·8$^{d}$ | 72·0$^{c,d}$ | 130·4$^{b,c}$ | 125·2$^{b,c}$ | 216·7$^{a,e}$ | 166·1$^{b,c}$ | 223·5$^{a,e}$ | 235·5$^{e}$ | 299·9$^{f}$ |
| (mg dL$^{-1}$) | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 25·8 | 17·8 | 5·1 | 5·3 | 3·8 | 15·1 | 13·1 | 10·4 | 17·0 | 19·0 | 10·9 | 16·3 |
| Protein secreted | 0·04$^{a}$ | 0·06$^{a}$ | 0·14$^{a,b,c}$ | 0·11$^{a,b}$ | 0·12$^{a,b}$ | 0·22$^{b,c}$ | 0·21$^{b,c}$ | 0·33$^{d,e}$ | 0·25$^{c,d}$ | 0·34$^{d,e}$ | 0·35$^{d,e}$ | 0·43$^{e}$ |
| (mg h$^{-1}$) | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 0·004 | 0·01 | 0·01 | 0·01 | 0·01 | 0·02 | 0·03 | 0·01 | 0·05 | 0·03 | 0·03 | 0·07 |
| Protease activity | 0·09$^{a,b}$ | 0·04$^{c}$ | 0·10$^{a,b,d}$ | 0·09$^{a,b}$ | 0·07$^{b,c}$ | 0·10$^{a,b,d}$ | 0·11$^{a,b,d}$ | 0·10$^{a,b,d}$ | 0·11$^{a,b}$ | 0·09$^{a}$ | 0·24$^{e}$ | 0·13$^{d}$ |
| (units h$^{-1}$) | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 0·02 | 0·01 | 0·02 | 0·01 | 0·01 | 0·02 | 0·01 | 0·01 | 0·03 | 0·01 | 0·06 | 0·02 |
| (units (mg protein)$^{-1}$) | 2·72$^{a}$ | 0·67$^{b,c}$ | 0·74$^{b,c}$ | 0·97$^{b}$ | 0·60$^{b,c}$ | 0·49$^{b,c}$ | 0·53$^{b,c}$ | 0·31$^{c}$ | 0·59$^{b,c}$ | 0·26$^{c}$ | 0·67$^{b,c}$ | 0·31$^{c}$ |
| | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 0·38 | 0·10 | 0·17 | 0·23 | 0·09 | 0·06 | 0·03 | 0·02 | 0·10 | 0·02 | 0·16 | 0·02 |
| No. of glands | 10 | 6 | 6 | 7 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 8 |

Means with different superscripts (a–f) are significantly different by Duncan's new multiple range test ($P < 0·05$).

on fluid and protein secretion by the salivary glands in response to substance P$^{Tyr8}$, a well-known analogue of substance P (Fisher & Folkers 1976).

The copious salivary flow and low concentrations of protein in the 25-day-old rats are also characteristic of salivas secreted by adult rats in response to cholinergic and peptidergic agonists (Schneyer & Hall 1968; Abe & Dawes 1978, 1982; Bobyock et al 1986). In the submandibular glands of 8-week-old rats, the salivary volumes and flow rates progressively increased with increasing doses in response to choline esters (Iwabuchi et al 1988), whereas in this report, in response to methacholine, they were significantly reduced at the highest dose tested. Thus it is suggested that methacholine (10 mg kg$^{-1}$) had toxic effects on the weanling rats and it would seem important that the optimal doses for maximum responses should be determined.

The concentrations of protein in salivas elicited by four choline esters were inversely related to salivary volumes secreted by submandibular glands of young adult rats (Iwabuchi et al 1988). In contrast, in the weanling rats they were not significantly changed over a wide range of doses except at a few of the doses used. The amounts of protein secreted progressively increased with increasing doses in the young adult rats (Iwabuchi et al 1988). In contrast, those in the weanling rats were relatively independent of dose. These discrepancies between adult and weanling rats suggest that during the weaning period the abilities for protein synthesis and storage in the acinar cells of salivary glands have not yet been fully developed.

Esteroprotease activities in rat submandibular salivas elicited by choline esters during the weaning period have not previously been studied. The relative activities of esteroprotease, which is characteristically located in the granular convoluted tubule cells (Kamogashira et al 1988; Tanaka et al 1990), were very low but increased significantly at high doses. These results suggest that during the weaning period the granular convoluted tubule cells had started to function weakly. Secretion by these cells is largely in response to $\alpha_1$-adrenoceptors (Matthews 1974), but they may be partly stimulated by choline esters via cholinergic receptors, as also shown in young adult rat submandibular glands (Iwabuchi et al 1988).

The volumes of saliva secreted and flow rates by submandibular glands during the weaning period in response to intraperitoneal injections of substance P were not significantly changed at the higher doses used. Similar results were also obtained in response to substance P$^{Tyr8}$. These results were consistent with previous reports on submandibular glands of adult rats in response to intravenous injections

Table 7. The effects of eledoisin-related peptide at different intravenous doses on submandibular salivary flow rates and protein secretion (means ± s.e.).

| | Dose (mg kg$^{-1}$) | | | |
|---|---|---|---|---|
| | 0·5 | 1 | 3 | 5 |
| Volume of saliva | 11·0$^{a}$ | 15·3$^{a}$ | 33·2$^{b}$ | 13·0$^{a}$ |
| ($\mu$L in 15 min) | ± | ± | ± | ± |
| | 1·8 | 1·3 | 2·9 | 1·1 |
| Flow rate | 2·85$^{a}$ | 3·61$^{a}$ | 8·17$^{b}$ | 3·11$^{a}$ |
| (nL mg$^{-1}$ min$^{-1}$) | ± | ± | ± | ± |
| | 0·43 | 0·25 | 1·16 | 0·26 |
| Protein concn | 569·3$^{a,b}$ | 504·3$^{b}$ | 344·7$^{b}$ | 819·0$^{a}$ |
| (mg dL$^{-1}$) | ± | ± | ± | ± |
| | 79·2 | 45·8 | 22·2 | 135·2 |
| Protein secreted | 0·06$^{a}$ | 0·07$^{a,b}$ | 0·11$^{c}$ | 0·10$^{b,c}$ |
| (mg 15 min$^{-1}$) | ± | ± | ± | ± |
| | 0·01 | 0·01 | 0·01 | 0·01 |
| Protease activity | 0·03$^{a}$ | 0·05$^{a,b}$ | 0·06$^{b}$ | 0.07$^{b}$ |
| (units 15 min$^{-1}$) | ± | ± | ± | ± |
| | 0·01 | 0·005 | 0·004 | 0·02 |
| (units (mg protein)$^{-1}$) | 0·37$^{a}$ | 0·54$^{b}$ | 0·56$^{b}$ | 0·77$^{b}$ |
| | ± | ± | ± | ± |
| | 0·10 | 0·07 | 0·05 | 0·15 |
| No. of glands | 8 | 9 | 9 | 4 |

Means with different superscripts (a–f) are significantly different by Duncan's new multiple range test ($P < 0·05$).

of substance P at different doses (Ekström et al 1983; Bobyock et al 1986).

Physalaemin, an endecapeptide originally extracted from the skin of the South American amphibian *Physalaemus fuscumaculatus*, is one of a group of vasoactive peptides classified as tachykinins, similar to analogues of substance P and eledoisin, on the basis of their homologous structures and physiological effects. There have been no previous reports, to our knowledge, on fluid secretion by submandibular glands of rats in response to physalaemin at different doses given intraperitoneally. With intravenous injections, physalaemin caused a copious flow of saliva by salivary glands of rats (Schneyer & Hall 1968) and dogs (Bertaccini et al 1965).

Although physalaemin is classified as a tachykinin, different results were obtained for the four tachykinins used here. Physalaemin at the doses of 15 or 20 $\mu$g kg$^{-1}$ was the most potent sialogogue of four tachykinins and three cholinergic agonists. It has already been reported that salivation in response to physalaemin was neither blocked nor modified in any way when cholinergic, $\alpha$- and $\beta$-adrenergic blockers were present (Emmelin & Lenninger 1967; Schneyer & Hall 1968). However, at doses (0·5, 1, 3 and 5 mg kg$^{-1}$) of eledoisin-related peptide used intraperitoneally, little salivation was observed. Thus we used eledoisin-related peptide for intravenous experiments only.

The protein concentrations and total outputs of protein secreted by the submandibular gland were very low in response to four tachykinins except for eledoisin-related peptide and resembled those with cholinergic stimuli, as shown previously in older rats (Yu et al 1983). However, the levels of both parameters tended to increase at high doses, as shown in adult rats in response to substance P (Bobyock et al 1986). The relative activities of esteroprotease in saliva elicited by four tachykinins were low and did not significantly increase at high doses except with physalaemin. These results are consistent with a report on arginine esterase (Fleming et al 1984) in saliva secreted by submandibular glands of adult rats in response to substance P, eledoisin and physalaemin. At the lowest doses, the specific activities of esteroprotease in saliva secreted in response to substance P, substance P$^{Tyr8}$ and physalaemin were higher as was seen in salivas elicited by methacholine and carbachol at the lowest or the highest doses. These data suggest that both types of agonists may also stimulate secretion of protease by the granular convoluted tubule cells via each receptor, which may not be yet clearly elucidated.

The protein type in saliva elicited by all agonists at all doses used here, and pilocarpine, were typical of the $\beta$-type. This also occurs in submandibular salivas elicited by $\beta_1$- and $\beta_2$-, and by low doses of almost all of the $\alpha_1$-adrenergic agonists in adult rats (Abe 1987). No special protein was observed in salivas elicited by an agonist over a wide range of doses. These results indicate that the mechanisms of fluid and protein secretion, as seen in adult rats, have already been established at the time of weaning.

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